

## CLAIMS LISTING

1 - 35 Cancelled

36 (New) A solid test strip designed for use in monitoring weight loss programs and capable of enabling a person to self-monitor weight loss on a daily basis in a sample of urine, saliva or other bodily fluid that is noninvasively obtainable, which provides a color signal, indicative of the  $\beta$ -hydroxybutyrate content of the sample upon being dipped in the sample, removed, allowed to rest briefly and read, which solid test strip comprises

- 1) an inert support layer and
- 2) a dried reagent layer comprising a porous material impregnated with
  - a)  $\beta$ -hydroxybutyrate dehydrogenase enzyme (" $\beta$ -HBD), which is either
    - (I) obtained from *Alcaligenes* or another source such that it is uninhibited by chloride ions or
    - (ii) is obtained from a source that is inhibited by chloride ions and is present in a concentration at least 10 to 20 times that used when the  $\beta$ -HBD is obtained from a source such that it is uninhibited by chloride ions,
  - b) nicotinamide dinucleotide ("NAD"),
  - c) a tetrazolium dye precursor
  - d) an electron mediator capable of transferring an electron to said dye precursor to effect a color change, and

e) a sufficient amount of a buffer having a pH in excess of 8.5 to maintain the reaction pH above 8.5 when the strip is saturated with urine or other bodily fluid.

37 (New) A solid test strip according to Claim 36 wherein the electron mediator is a diaphorase enzyme.

38 (New) A solid test strip according to Claim 36 wherein the tetrazolium dye precursor is nitrobluetetrazolium (“NBT”) or 2-(indophenyl)-3-(paranitrophenyl)-5-phenyl tetrazolium chloride (“INT”).

39 (New) A solid test strip designed for use in monitoring weight loss programs and capable of enabling a person to self-monitor weight loss on a daily basis in a sample of urine, saliva or other non-invasively obtainable bodily fluid, which provides a color signal indicative of the combined  $\beta$ -hydroxybutyrate and acetoacetate content of the sample upon being dipped in the sample, removed, allowed to rest briefly and read, which solid test strip comprises

- 1) an inert support layer and
- 2) a dried reagent layer comprising a porous material impregnated with
  - a)  $\beta$ -HBD enzyme, which is either
    - (i) obtained from *Alcaligenes* or another source such that it is uninhibited by chloride ions or

(ii) is obtained from a source that is inhibited by chloride ions and is present in a concentration at least 10 to 20 times that used when the  $\beta$ -HBD is obtained from a source such that it is uninhibited by chloride ions,

- b) NAD
- c) a tetrazolium dye precursor
- d) an electron mediator capable of transferring an electron to said dye precursor to effect a color change and
- e) a sufficient quantity of a buffer having a pH of at least 7.0 but less than 8.5, to maintain the reaction pH below 8.5 but not less than about 7.0 when the strip is saturated with urine or other bodily fluid.

40 (New) A solid test strip according to Claim 39 wherein the electron mediator is a diaphorase enzyme.

41 (New) A solid test strip according to Claim 39 wherein the tetrazolium dye precursor is NBT or INT.

42 (New) A solid test strip designed for use in monitoring weight loss programs and capable of enabling a person to self-monitor weight loss on a daily basis in a sample of urine, saliva or other non-invasively obtainable bodily fluid, which provides a color signal indicative of the combined  $\beta$ -hydroxybutyrate and acetoacetate content of the sample upon being dipped in the sample, removed, allowed to rest briefly and read, which solid test strip comprises

- 1) an inert support layer and
- 2) a dried reagent layer comprising a porous material impregnated with
  - a)  $\beta$ -HBD enzyme, which is either
    - (i) obtained from *Alcaligenes* or another source such that it is uninhibited by chloride ions or
    - (ii) is obtained from a source that is inhibited by chloride ions and is present in a concentration at least 10 to 20 times that used when the  $\beta$ -HBD is obtained from a source such that it is uninhibited by chloride ions,
  - b) NAD,
  - c) a nitroprusside salt or a diazonium salt in a quantity sufficient to react with both endogenous acetoacetate obtained by conversion thereto of  $\beta$ -hydroxybutyrate in the sample, and
  - d) a sufficient quantity of a buffer have a pH about 8.5 or higher to maintain the strip at the same pH when saturated with sample.
- 43 (New) A solid test strip according to Claim 42 wherein the electron mediator is a diaphorase enzyme.
- 44 (New) A solid test strip according to Claim 42 wherein the tetrazolium dye precursor is NBT or INT.
- 45 (New) A test strip according to Claim 42 wherein ingredient (c) is sodium nitroprusside.
- 46 (New) A test strip according to Claim 42 wherein ingredient (c) is a diazonium salt.

47 (New) A test strip according to Claim 46 wherein ingredient (c) is 4-nitrobenzenediazonium fluoborate.

48 (New) A solid test strip designed for use in monitoring weight loss programs and capable of enabling a person to self-monitor weight loss on a daily basis in a sample of urine, saliva or other bodily fluid that is noninvasively obtainable, which provides a color signal, indicative of the total ketone bodies content of the sample upon being dipped in the sample, removed, allowed to rest briefly and read, which solid test strip comprises

- 1) an inert support layer and
- 2) a dried reagent layer comprising a porous material impregnated with
  - a)  $\beta$ -HBD
  - b) NAD
  - c) a nitroprusside salt or a diazonium salt in sufficient quantity to
    - (i) immediately react with the acetone present in the sample and stabilize it against volatilization and
    - (ii) also react with the endogenous acetoacetate in the sample and with acetoacetate obtained by the conversion thereto of  $\beta$ -hydroxybutyrate in the sample and
  - d) a sufficient quantity of a buffer having a pH of about 8.5 or higher to maintain the reaction pH at the same level when the strip is saturated with sample.

49 (New) A solid test strip according to Claim 48 wherein the electron mediator is a diaphorase enzyme.

50 (New) A solid test strip according to Claim 48 wherein the tetrazolium dye precursor is NBT or INT.

51 (New) A test strip according to Claim 48 wherein ingredient (c) is sodium nitroprusside.

52 (New) A test strip according to claim 48 wherein ingredient (c) is a diazonium salt.

53 (New) A test strip according to Claim 51 wherein ingredient (c) is 4-nitrobenzene-diazonium fluoborate.

54 (New) A method for monitoring the level of  $\beta$ -hydroxybutyrate present in a sample of urine or another human bodily fluid that can be noninvasively obtained, which comprises contacting said sample with a mixture comprising the following ingredients:

- a)  $\beta$ -HBD which either
  - (i) has been obtained from *Alcaligenes* or another source such that it is not inhibited by chloride ions, or else
  - (ii) has been obtained from a source such that it is inhibited by chloride ions and is present in an excess amount from 10 to 20 times the concentration utilized when the  $\beta$ -HBD is not inhibited by chloride ions,
- b) NAD
- c) a tetrazolium dye precursor,

- d) an electron mediator and
- e) a buffer having a pH above 8.5

and measuring by electrochemical, spectrophotometric or fluorometric means, or by comparison of the develop color to a preestablish color intensity standard, the amount of  $\beta$ -hydroxyrate in the sample.

55 (New) A method according to Claim 54 wherein the tetrazolium dye precursor is NBT or INT.

56 (New) A method according to Claim 54 wherein the electron mediator is a diaphoruse enzyme.

57 (New) A method for monitoring the level of combined acetoacetate and  $\beta$ -hydroxybutyrate in a sample of human bodily fluid which comprises contacting the sample with a mixture of the following ingredients:

- a)  $\beta$ -HBD
- b) NAD
- c) a tetrazolium dye precursor,
- d) an electron mediator, and
- e) a buffer having a pH that is over 7.0 but less than 8.5,

and measuring by electrochemical, spectrophotometric or fluorometric means, or by comparison of the color developed to a preestablished color intensity standard, the combined amount of  $\beta$ -hydroxybutyrate and acetoacetate present in the sample.

58 (New) A method according to Claim 57 wherein the sample is urine or another fluid that can be noninvasively obtained and the  $\beta$ -HBD is either (I) obtained from *Alcaligenes* or another source such that it is not inhibited by chloride ions, (ii) or else has been obtained from a source such that it is inhibited by chloride ions and is present in an excess amount from about 10 to 20 times the amount utilized when the  $\beta$ -HBD is not inhibited by chloride ions.

59 (New) A method according to claim 57 wherein the tetrazolium dye precursor is NBT or INT.

60 (New) A method according to claim 57 wherein the electron mediator is a diaphorase enzyme

61 (New) A method for monitoring the level of combined acetoacetate and hydroxybutyrate in a sample of human bodily fluid which comprises contacting said sample with a mixture comprising the following ingredients:

- a)  $\beta$ -HBD,
- b) NAD,
- c) a nitroprusside salt or a diazonium salt in a quantity sufficient to react with endogenous acetoacetate in the sample and acetoacetate obtained by conversion thereto of  $\beta$ -hydroxybutyrate in the sample, and
- d) a buffer having a pH of about 8.5 or higher

and measuring by electrochemical, spectrophotometric or fluorometric means, or by comparison of the color developed to a preestablished color intensity standard, the amount of combined acetoacetate and  $\beta$ -hydroxybutyrate in the sample.

62 (New) A method according to Claim 61 wherein the sample is urine or another fluid that can be noninvasively obtained and the  $\beta$ -HBD is either (i) obtained from *Alcaligenes* or another source such that it is not inhibited by chloride ions, or else (ii) has been obtained from a source such that it is inhibited by chloride ions and is present in an excess amount from about 10 to 20 times the amount utilized when the  $\beta$ -HBD is not inhibited by chloride ions.

63 (New) A method according to Claim 31 wherein the tetrazolium dye precursor in NBT or INT.

64 (New) A method according to Claim 31 wherein the electron mediator is a diaphorase enzyme.

65 (New) A method according to Claim 61 wherein ingredient (c) is a nitroprusside salt.

66 (New) A method according to Claim 61 wherein ingredient (c) is a diazonium salt.

67 (New) A method according to Claim 66 wherein ingredient (c) is 4-nitrobenzene diazonium fluoborate

68 (New) A method for monitoring the level of total ketone bodies in a sample of human bodily fluid which comprises contacting said sample with a mixture comprising the following ingredients:

- a)  $\beta$ -HBD,
- b) NAD,
- c) a nitroprusside or diazonium salt in an amount sufficient to
  - (i) react instantaneously with and stabilize acetone in the sample,
  - (ii) also react with endogenous acetoacetate in the sample and

(iii) also react with acetoacetate formed by conversion thereto of  
β-hydroxybutyrate in the sample, and

d) a buffer having a pH of about 8.5 or higher,

and measuring by electrochemical, spectrophotometric or fluorometric means, or by comparison of the color developed to a preestablished color intensity standard the amount of total ketone bodies in the sample.

69 (New) A method according to Claim 68 wherein the sample is urine or another fluid that can be noninvasively obtained and the β-HBD is either (i) obtained from *Alcaligenes* or another source such that it is not inhibited by chloride ions, or else (ii) has been obtained from a source such that it is inhibited by chloride ions and is present in an excess amount from about 10 to 20 times the amount utilized when the β-HBD is not inhibited by chloride ions.

70 (New) A method according to claim 68 wherein the tetrazolium dye precursor is NBT or INT.

71 (New) A method according to claim 68 wherein the electron mediator is a diaphorase enzyme.

72 (New) A method according to claim 68 wherein ingredient (c) is a nitroprusside salt.

73 (New) A method according to claim 68 wherein ingredient (c) is a diazonium salt.

74 (New) A method according to claim 73 wherein ingredient (c) is 4-nitrobenzene diazonium fluoborate.